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PROGRAM
AND ABSTRACTS
OF PAPERS
CITRUS
RESEARCH
CONFERENCE

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FRUIT AND VEGETABLE
CHEMISTRY LABORATORY
263 SOUTH CHESTER AVENUE
PASADENA CALIFORNIA 91106

Western Marketing and Nutrition Research Division
Agricultural Research Service

UNITED STATES DEPARTMENT OF AGRICULTURE

FOREWARD

This Citrus Research Conference is being held to bring to members of the citrus and allied industries in Southern California and Arizona the latest results of research on the chemistry, pharmacology, and technology of citrus fruits and their products carried on by the Marketing and Nutrition Research Divisions of the Agricultural Research Service, U.S. Department of Agriculture. The following are participating in this year's conference.

Western Marketing and Nutrition Research Division:

Western Regional Research Laboratory (Division headquarters), Berkeley, Calif. 94710

Fruit and Vegetable Chemistry Laboratory
263 South Chester Avenue, Pasadena, Calif. 91106

Southeastern Marketing and Nutrition Research Division:

Citrus and Subtropical Products Laboratory
600 Avenue S, N.W., Winter Haven, Florida 33882

Market Quality Research Division:

Horticultural Crops Branch
P. O. Box 700, Pomona, Calif. 91769

Conference headquarters: Huntington-Sheraton Hotel 1401 South Oak Knoll Ave. Pasadena, California

THE USE OF INDIVIDUAL AMINO ACIDS IN THE ESTIMATION OF ORANGE JUICE CONTENT*

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The problem of determining orange juice content or authenticity is of great importance both nutritionally and economically to the consumer and to the citrus industry. Furthermore, the widely discussed labeling standards for juice content in orange juice products are dependent upon adequate analytical procedures for practical enforcement.

The approach to solving the problem, as it is being investigated in this laboratory, involves three phases. The first phase, which is essentially complete, was to measure the concentrations of many of the constituents in a representative sampling of juices. The second phase, well underway, is selecting and evaluating those constituents which have high correlations among themselves and with juice content. The third phase, which is just beginning, will be to develop and test equations, simplify procedures, and verify results with multiple samples.

Several parameters investigated in this laboratory appear to be significantly correlated among themselves and with juice content. These include the Brix-acid ratio, total amino acids, total phenolics, citric acid, γ -aminobutyric acid and aspartic acid. These correlations and regression equations will be presented and discussed. The relationships for estimating juice content are based on parameters which are unaffected by dilution with sugar, acid and/or water. The compounds involved are also too expensive to be economically added to the juice for purposes of sophistication.

Batch type ion exchange separations of γ -aminobutyric acid, total aspartic acid, and other key amino acids have been developed. These simplified procedures would make practical the routine analysis of these amino acids in control laboratories for purposes of determining juice content of orange juice products.

*This research was supported in part by the Lemon Products Technical Committee, Los Angeles, California.

QUANTITATIVE AND QUALITATIVE ANALYSES OF SOME CITRUS ESSENTIAL OILS

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Distilled Valencia orange and grapefruit essence oils were quantitatively analyzed for their main constituents by gas chromatography. These essence oils are the oily layer separated from the aqueous layer during commercial preparation of aqueous essence. Approximately 15 compounds from each oil were identified and their percentages in the oils determined from peak areas obtained from their gas chromatograms.

These essence oils were fractionally distilled at 0.5-1.0 mm. pressure for qualitative analytical studies. The most volatile fraction of either oil was condensed at liquid nitrogen temperature and was therefore separated from the bulk of the distillate, D-limonene, that was condensed at chilled water temperature. From both Valencia orange and grapefruit essence oils, the highly volatile distillate trapped at liquid nitrogen temperature represented less than 1 percent by weight of the oil but possessed most of the essence-like odor of the starting essence oil. Qualitative analysis of this volatile fraction from Valencia orange essence oil (table 1) afforded 22 identified components including two

Table 1.--Composition of Valencia orange essence oil volatile fraction

Compound	Weight percent	Compound	Weight percent	Compound	Weight percent
Hexane	0.28	Ethyl vinyl ketone	0.095	Linalool	0.104
Heptane	.38	Ethyl butyrate	2.874	Carvone	.534
Acetone	.46	α -Pinene	2.421	t-Carveol	.374
Ethyl acetate	.122	Hexanal	.850	c-Carveol	.165
Acetal	.064	Sabinene	.405	t-2,8-p-mentha- diene-1-ol	.441
Ethanol	2.474	Myrcene	.120	c-2,8-p-mentha- diene-1-ol	.448
Ethyl propionate	.036	D-Limonene	85.821		
Methyl butyrate	.202	Octanal	.446		

new citrus constituents, ethyl vinyl ketone and ethyl propionate. This fraction from Valencia orange essence oil was shown by an odor panel to be necessary for the original oil to have a full essence-like odor. From grapefruit essence oil 19 components including one new citrus constituent, ethoxymethoxyethane, were identified.

Quantitative analysis of the highly volatile fraction from Valencia orange essence oil was carried out by using a gas-chromatograph-digital integrator combination. A synthetic mixture based on this analysis was

prepared to determine response factors for correcting peak areas for each component. Table 1 lists weight percent of each component in the volatile fraction as calculated by using the response factors. Based on these weight percent values, a second synthetic mixture was prepared and evaluated. An odor panel found its odor to be closer to the natural fraction than that of the first synthetic mixture.

Cold-pressed citrus oils are not as amenable to quantitative analysis by gas liquid chromatography (GLC) as are the distilled essence oils, because of the waxes, carotenoids and other high-boiling components present in the cold-pressed oils. For meaningful quantitative GLC results with cold-pressed citrus oils, the quantity of nonvolatile material present must be determined. Furthermore, some means of removing this nonvolatile material from the GLC column must be available if a column is to be used for repeated analyses of oil samples. A technique for rapidly removing high-boiling materials from a nonpolar GLC column is now available; it involves injecting a small sample of a silanizing agent into the column at the end of a run at the maximum operating temperature and maintaining that temperature for 5-10 minutes. By this procedure nonvolatile substances are removed, a drifting base-line is restored to a normal level, and column resolution is also restored. This technique has been utilized to quantitatively analyze cold-pressed Persian lime oil by the following procedure.

Cold-pressed lime oil was quantitatively analyzed by GLC and weight percent of each identified component present was calculated after GLC response factors had been determined for each component. A sample of cold-pressed lime oil was distilled at low pressure to determine the percent high-boiling material present that would not be eluted from the GLC column during the analysis period. Correcting for the percent nonvolatiles present, actual weight percent of each identified component present in the cold-pressed oil was then determined as listed in table 2.

Table 2.--Quantitative composition of Persian lime oil

Compound	Weight percent	Compound	Weight percent
α -Pinene	2.44	Geranial	5.08
β -Pinene	11.90	Neryl acetate +	3.05
D-Limonene	47.75	Geranyl acetate	
γ -Terpinene	16.26	β -Caryophyllene +	1.51
Terpinolene	.63	α -Bergamotene	
Neral	3.16	β -Bisabolene	2.47

With these quantitative data, a synthetic mixture with a lime-like aroma was prepared. However, odor panel evaluation showed it to be distinguishable from natural lime oil, even when the proper amount of lime oil nonvolatiles (not accounted for by GLC analysis) was added to the synthetic mixture. The quantitative analytical technique applied here to Persian lime oil is applicable to other cold-pressed citrus oils as well.

METABOLIC DEBITTERING:
A METHOD FOR REDUCING LIMONIN BITTERNESS OF CITRUS JUICES*

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Despite the overall high quality of the citrus crop, portions of the crop tend to yield juice whose quality suffers because of limonin-caused bitterness. While limonin bitterness has historically been associated with navel orange juice, it is now recognized that the juice of other varieties of oranges as well as that from lemons, grapefruit, and tangerines is on occasion subject to limonin bitterness. This type of bitterness is unique in that it generally develops after the juice is extracted from the fruit. In earlier work we have shown that the change in taste is caused by the conversion of a nonbitter substance (limonoate A-ring lactone) to a bitter substance (limonin) after the juice is extracted from the fruit. The non-bitter substance occurs naturally in the fruit tissues where it is stable and remains nonbitter. However, when the fruit tissues are broken during juice extraction this normally nonbitter substance is attacked by the juice acids and is converted into the intensely bitter limonin; juice containing as little as 6 p.p.m. is bitter.

Up to the present time no practical method has been found to remove limonin from juice. However, research on its chemistry and biochemistry over the past several years has opened two promising new approaches to the problem, namely, metabolic debittering and enzymatic debittering. (see Hasegawa et al. and Bennett et al. elsewhere in this Program for reports of the enzymatic debittering approach).

Studies on the biochemistry of limonin led to the observation of a metabolic system in the intact orange that functions while the fruit is still on the tree to prevent bitterness from developing later in the juice. However, this system has its effect late in the harvest season after most of the navel orange crop has been harvested; it acts to prevent bitterness by destroying the nonbitter precursor substance. Based on these observations it was reasoned that the juice bitterness problem could be solved if a way could be found to activate this natural metabolic system at an earlier stage of fruit maturity. In biochemical language an agent was needed to "turn-on" the system.

A search for such agents is underway. One agent, which we reported last year, is 2-chloroethylphosphonic acid (CEPA). We found that within 5 days after the fruit has been dipped in a dilute water solution of CEPA, the limonin content of the extracted juice is 20 to 40 percent lower than that of juice from untreated fruit. Taste tests confirm that the treatment results in juice of substantially reduced bitterness that retains the desirable flavor characteristics of fresh orange juice.

*This research was supported in part by the Citrus Advisory Board, Los Angeles, California.

While CEPA appears to have considerable promise it is not yet certain whether it will eventually be adopted by the citrus industry. Before it can be used commercially CEPA must be thoroughly tested and proven safe for use on foods, as required by the FDA. Such tests are currently being conducted by the manufacturer.

In the meantime we are continuing our search for other agents, based on what we have learned about the mechanism for "turning-on" the debittering metabolic system. We now know that CEPA acts through a specific ethylene mechanism which appears unrelated to the general effects this substance has on plants (Citrograph 56(11): 351, 1971). Prolonged, accelerated respiration, a common ethylene effect, is not required for the debittering metabolic system to function. In fact, exposure of the fruit to low concentrations of ethylene for as brief a period as 3 hours is sufficient to trigger the metabolic debittering system. Thus, navel oranges treated with 20 p.p.m. ethylene for 3 hours followed by a 5-day holding period without ethylene produced juice having 32 percent lower limonin content than the juice from untreated fruit. In the same experiment CEPA caused a similar reduction in juice limonin levels. Exposure of the fruit to ethylene for the entire 5-day period gave lower quality juice without decreasing limonin below 3-hour treatment levels.

The triggering of metabolic debittering by brief exposure of the fruit to ethylene is a new observation that has considerable commercial potential. Further work is in progress to determine the optimum conditions of treatment. However, there appears to be no reason why commercial application of a treatment, involving exposure of fruit for several hours to 20 p.p.m. ethylene, removal from gassing room, and holding for about 5 days before juicing, could not begin on a trial basis.

Other research on limonin bitterness is being conducted by Hasegawa and Bennett at our laboratory. The objective of their work is to produce a specific enzyme which can be added to bitter juice to destroy limonin. It is envisioned that success in both approaches will lead to a two-pronged attack whereby accelerated metabolism greatly reduces the amount of limonin entering the juice, and the debittering enzyme destroys any which does enter.

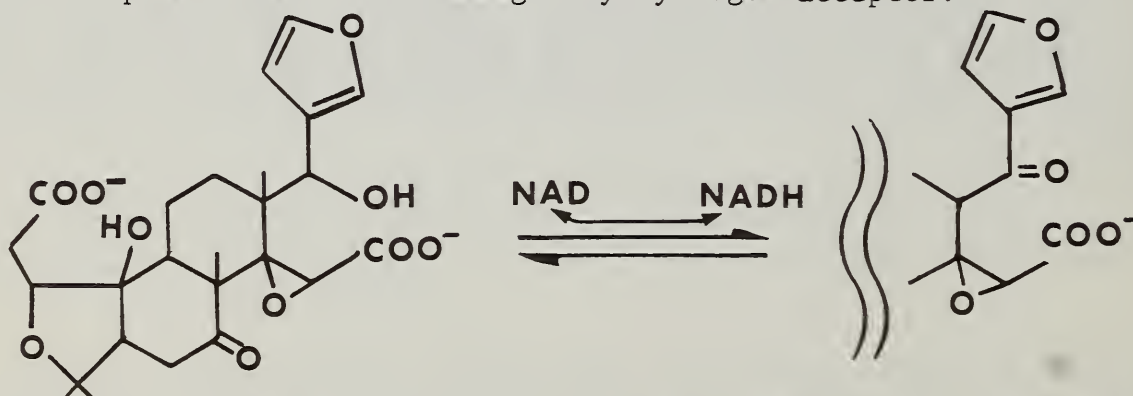
LIMONOATE DEHYDROGENASE, A DEBITTERING
ENZYME FROM *ARTHROBACTER GLOBIFORMIS*

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In dealing with the problem of bitterness in citrus juices due to limonin, one of the approaches taken at the Pasadena Laboratory is to investigate the possibility of converting limonin to nonbitter compounds by treating the juice during extraction and processing with a limonin debittering enzyme. Since microorganisms are known to be a potential source of many enzymes, we have been investigating that source for a limonin-degrading enzyme.

During a recent survey of microorganisms, we isolated a pure culture of the bacterium, *Arthrobacter globiformis* which grows well on a medium containing Na-limonate as the only carbon source. Analyses of metabolites of limonoate present in the bacterial growing medium just before reaching the maximum stationary phase showed that approximately 80 percent of the metabolized limonoate was a dehydrogenation product which has been named 17-dehydrolimonoate. It was also found that 17-dehydrolimonoate and 17-dehydrolimonoate A-ring lactone are nonbitter compounds.

Since we observed large amounts of 17-dehydrolimonoate in the medium, it was reasonable to believe that this compound is the first metabolite of limonoate produced by *Arthrobacter globiformis*. An enzyme responsible for the conversion of limonoate to nonbitter dehydrolimonoate was found in cell-free extracts of the bacterium. Preliminary tests showed that this new enzyme, limonoate dehydrogenase, has its optimum activity at pH 9.5 and requires NAD as the obligatory hydrogen acceptor.



The dehydrogenase was partially purified by ammonium sulfate precipitation followed by DEAE cellulose column chromatography. Substrate specificity studies of the purified enzyme indicated that changes in the structure of the A-ring of limonoate gave compounds which were attacked more rapidly by the enzyme, whereas the removal of either the epoxide or furan ring resulted in compounds which were completely resistant to attack.

Also, it seems likely that in order to be a substrate the limonoid compound must have an open D-ring. Tests of the effects of inhibitors and activators showed that zinc ions and sulfhydryl groups are essential for dehydrogenase activity.

Studies are now underway to determine the conditions under which limonoate dehydrogenase is capable of debittering citrus juices. In reconstituted navel orange concentrate adjusted to the pH optimum of the enzyme, essentially all of the limonoate was converted to dehydrolimonoate. At a slightly acidic pH the activity of the enzyme was lower but substantial conversion of limonoate could still be achieved. While it appears that upward adjustment of juice pH is necessary in order for limonoate dehydrogenase to catalyze the debittering reaction, a number of avenues bearing on the extent of the adjustment remain to be studied. One of these avenues, namely matrix-bound and immobilized enzymes, also has potential application in the development of a continuous commercial enzymic debittering process. In any event, this work shows for the first time the presence of a soluble limonoid decomposing enzyme in microorganisms and demonstrates the feasibility of this approach to debittering citrus juices. Work aimed at isolating other limonin-degrading enzymes is in progress.

ISOLATION AND STRUCTURE DETERMINATION OF BACTERIAL METABOLITES OF LIMONIN

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As reported elsewhere in this Program we have been engaged in a search for microorganisms which metabolize the citrus bitter principle limonin to nonbitter products. Hopefully, enzymes obtained from such organisms could be used to debitter citrus juices. Once we had found several bacterial cultures which grew on a medium containing limonin as a sole carbon source, it was necessary to determine the nature of the metabolites. This report deals with the approach and methods used to characterize the compounds produced by one organism.

From a mixed culture of soil bacteria which grew well on a limonin-containing medium, a single organism was isolated and identified as *Arthrobacter globiformis*. This bacterium was incubated on a large scale with limonin, and after three days the products were isolated by extraction. The extract was separated into neutral and acidic fractions. Thin-layer chromatography (TLC) of the neutral fraction showed that it consisted almost entirely of unchanged limonin. All of the metabolites were found in the acidic fraction, which was methylated to facilitate separation of its components by chromatography. TLC of this fraction showed one major constituent and several lesser ones. The main compound was isolated by crystallization and identified by its infrared and nuclear magnetic resonance (NMR) spectra as methyl 17-dehydrolimonoate A-ring lactone. The dehydrogenation reaction producing this nonbitter metabolite represents the first step in the degradation of limonin by this organism.

We then proceeded to isolate as many of the other metabolites as possible, first by column chromatography and then by preparative TLC. Most of these compounds were very similar in their chromatographic behavior, and five or six different separations were usually necessary to obtain an individual component in pure form. We have thus far isolated fifteen of these metabolites, and they seem to fall into two general classes representing separate metabolic pathways. In one case the furan ring is retained and the A and B rings of limonin are degraded. Of the metabolites which we have isolated, the most highly degraded product of this pathway is methyl 1,3-dimethyl-2,6-diketo-3-(β -furoyl)cyclohexanecarboxylate. In the second pathway, the furan ring is apparently removed at an early stage, followed by changes at various positions in the molecule.

During the course of this work we have made extensive use of NMR spectroscopy in attempting to determine the structures of these metabolites. In some cases a conventional NMR spectrum provides sufficient information, but frequently limonoid spectra are so complex that unambiguous interpretation is impossible. We have therefore applied a new technique, the use of paramagnetic shift reagents, to clarify our limonoid NMR spectra.

These shift reagents spread out the spectra and thereby allow formerly overlapping signals to be seen individually. We have further discovered that the usefulness of this technique can be greatly increased by varying the temperature of the samples. Examples will be shown of the applications of this method for clarification of NMR spectra of limonoids.

A NEW SYSTEM FOR IMMOBILIZING ENZYMES FOR PROCESSING LIQUID PRODUCTS

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Where applicable, the ultimate refinement in juice processing technology lies in the utilization of enzymes to accomplish desired transformations. Enzymes are the agents involved in natural processes by which plants and organisms generate the food we eat, and enzymes are consumed as constituents of foods. Most enzymes are quite selective in activity, are less prone to cause detrimental side effects than chemical reagents, and operate with high efficiency at moderate temperatures.

In the soluble form, however, enzymes react rather slowly, undergo heat denaturation and proteolytic degradation, and are often subject to product inhibition. Their action can be terminated only by destructive heating, and the enzyme is not recovered but consumed in the product.

On the other hand, immobilization of enzymes makes it possible to remove enzyme from the product. Contact time, and thereby the extent of reaction, can be precisely controlled. Immobilized enzymes can be used in continuous operations either packed in columns and in stirred flow-through reactors or in batch operations from which they can be recovered and reused. Product inhibition is reduced in column operation. In many examples, especially where the substrate is a small molecule, reaction can be very rapid with high efficiency.

For the past ten years or so, investigations on systems for immobilizing enzymes have been conducted primarily to study the basic mechanisms of enzyme action under conditions simulating conditions in living cells (Silman and Katchalski, *Ann. Rev. Biochem.* 35:873, 1966). With the development of successful systems, scientists have now begun to consider the application of these in analytical procedures and to medicine and processing. So far, their actual use in processing has been limited because of the complexity and expense of the systems used for insolubilization and the need to use purified enzymes.

We are currently working with a simple, inexpensive procedure based on a recent report by Negoro in Japan (*J. Ferment. Technol.*, Japan 48: 689, 1970). Negoro precipitated invertase from solution with tannic acid, mixed the precipitate with filter aid, and used it in a column.

With lactase enzyme, we found that when the precipitate was centrifuged down and resuspended in fresh water a number of times, it gradually disappeared, and considerable activity was found in the supernatant even after as many as ten washings. However, we found that the

enzyme could be fixed in the complex by treating with glutaraldehyde. The combined tannic acid-glutaraldehyde system uses cheap chemicals, is simple, and can be effective with relatively impure enzyme preparations. We find it is better to include filter aid in the initial tannic acid precipitation step to reduce the tendency to separate into separate phases in the column in slurry packing. This system has been successfully applied to glucose oxidase, invertase, peroxidase, catalase, α -chymotrypsin, glucoamylase, β -galactosidase (lactase) and other enzymes. See table 1 for data on retention of activity.

Table 1.--Enzymes immobilized with tannic acid-glutaraldehyde.
Comparison of activity with soluble enzymes.

Enzyme	Percent of soluble form ¹
Glucose oxidase ("Dee-0")	57
Invertase	55
Peroxidase	20
Catalase	52
α -Chymotrypsin	n.d. ²
Glucoamylase	15
β -Galactosidase (lactase)	69

¹ Weight basis not necessarily under optimum conditions.

² n.d. = not determined.

In an extension of Goldman's (Science 150:758, 1965) collodion membrane system for papain, we have found that glutaraldehyde will permanently fix enzyme embedded in collodion films. Goldman used bis-diazo-benzidine 2,2'-disulfonic acid as cross-linking agent. With glutaraldehyde-collodion we have obtained active, permanently fixed enzyme with papain, α -chymotrypsin, lactase, and peroxidase. This system could be used to coat screening or the surface of baffles exposed to liquid products.

The tannic acid-glutaraldehyde immobilized enzymic material is a very finely divided powder which is suitable only for processing clarified liquids. Consequently, a similar material was sought which would be equally cheap and available in granular form. We found that a phenolic resin (Duolite S-30, Diamond Shamrock Chemical Co.) could be used. The enzyme in aqueous solution is stirred gently with an appropriate amount of resin, a suitable amount of aqueous glutaraldehyde is added, and the mixture allowed to stand (with gentle swirling) overnight in the cold room. The aqueous phase is filtered off, and the resin is thoroughly washed to remove reagents and then packed in a column. We have been using 10- to 40-mesh material in up-flow mode, which is satisfactory for fluids containing suspended material, e.g., milk and citrus juices. Data will be presented on operating parameters for invertase, glucose oxidase, and lactase insolubilized with S-30 phenolic resin and glutaraldehyde.

RECENT DEVELOPMENTS IN THE HANDLING AND STORAGE OF FRESH CITRUS

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Projections of citrus production indicate sizeable increases in all categories of fruit by 1975. Even with a major part of this increase utilized by processing, the quantity available for fresh consumption will increase. It is the goal of the Market Quality laboratories to provide fresh citrus fruits of high quality with minimum decay for both increased consumption in the domestic market and increased export to Europe and to the Orient.

Storage of oranges at shipping point is used principally to keep an adequate supply of fruit on hand for marketing. Long storage is never desirable since the flavor of fresh oranges is best at the time of harvesting. Work done on the controlled atmosphere storage of California oranges had not been promising because of the loss of flavor and increased decay.

Lemons are stored to a greater extent than oranges since a large portion of the lemon crop is picked during a period of low consumption, and lemons are usually allowed to color during storage. Fortunately, lemons store better than oranges and, with proper attention to humidity, may be stored six months or longer.

Both oranges and lemons are often degreened with ethylene to obtain optimum color for marketing. Considerable work has been done on this operation since it is generally considered that ethylene weakens the fruit and makes it more subject to physiological injury and decay.

The export market is an important and profitable outlet for California fresh citrus. Considerable effort has been devoted to insuring the arrival in foreign ports of a high quality product. Much of this work has been done on determining the proper usage of the decay inhibitors, biphenyl and thiabendazole.

STRUCTURE-ACTIVITY RELATIONS IN DIHYDROCHALCONE SWEETENERS

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Since 1961, when the first sweet dihydrochalcone was prepared, many variations in structure have been tried. Most of the variations have centered upon the hydroxyl and alkoxy groups of the B-ring because it is relatively easy to make alterations in that part of the molecule. Depending on the substitution pattern in the B-ring, the compounds can be divided into the following four groups: (I) mono- or polyhydroxy substituted; (II) hydroxy-alkoxy disubstituted; (III) hydroxy-alkoxy trisubstituted and (IV) nonhydroxy substituted. The structures of the compounds and their tastes are shown in the table.

Table 1.--Structure and taste of substituted dihydrochalcones

Compound	Substituent		Present	At	Taste ¹
	2	3	4	5	
I					
1			OH		+++
2		OH			+++
3	OH				-
4		OH	OH		+
5		OH	OH	OH	nil
II					
6		OH	OMe		+++
7		OH	OEt		+++
8		OH	O-n-Pr		++++
9		OH	O-i-Pr		++
10	OH	OMe			++
11		OMe	OH		nil
12		OEt	OH		nil
III					
		OH	OMe	OH	nil
	MeO	OH	OMe		nil
IV					
13			OMe		- +
14		OMe	OMe		- +
15		Me	OMe		nil

¹ + signifies sweet; - signifies bitter.

The data for the four groups of compounds allow several conclusions to be made about structure-activity relations:

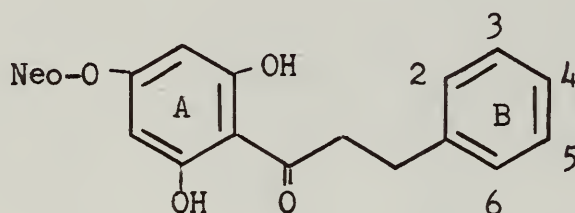
1) A hydroxy group is necessary for sweetness but its presence does not guarantee it.

2) The absence of a hydroxy group assures nonsweetness or bitter-sweetness.

3) For sweetness to subsist in hydroxy-alkoxy disubstituted compounds the order of the groups must be R-H-OH-alkoxy or R-OH-alkoxy (R = neo-O-Ar-COCH₂CH₂-).

4) Taste is abolished if the order of groups in hydroxy-alkoxy disubstituted compounds is R-H-alkoxy-OH. It is also abolished if three adjacent groups are present in addition to R.

These results can be rationalized in terms of a hypothetical taste receptor, which will be discussed. The results might also serve as a guide in predicting the taste of new compounds in this and related series.



INDUCED COLOR AND PROVITAMIN A CHANGES
IN NAVEL ORANGE AND MARSH SEEDLESS GRAPEFRUIT

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and

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At this meeting a year ago we reported that 2-(4-chlorophenylthio) triethylamine hydrochloride (CPTA) caused a major alteration of citrus color. In continuing these studies we investigated the effect of CPTA on the color of two citrus fruits, the Marsh seedless grapefruit and the Washington navel orange. These citrus fruits exhibit dissimilar patterns of color development during maturation. In the grapefruit, net synthesis or accumulation of carotenoid pigments ceases when the decrease in chlorophyll occurs, resulting in a relatively low concentration of pigments and light-colored (yellow) fruit. In contrast, loss of chlorophyll in the ripening navel orange is accompanied by the rapid accumulation of carotenoid pigments, thus resulting in a much higher concentration of carotenoids and consequently a much deeper color (orange). After treatment with CPTA and subsequent prolonged storage, both citrus fruits developed the same degree of intense red color, much like that of a ripe red variety of tomato. By altering the treatment technique, a uniform and desirable deep orange color developed overnight in the navel orange. Examination of the pigment constituents of these treated fruits showed the accumulation of lycopene in major amounts accompanied by a significant synthesis of the provitamin A carotene, γ -carotene. CPTA, however, does not appear to have any effect on the important terpenoid flavoring constituents which are found in the peel oil. These results and their implications with regard to improving the color and provitamin A content of citrus fruits will be considered in detail.

THE MODE OF ACTION OF CPTA IN CAROTENOGENESIS
AND ITS IMPORTANCE IN COLOR AND PROVITAMIN A ENHANCEMENT OF CITRUS FRUITS

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The compound 2-(4-chlorophenylthio) triethylamine hydrochloride (CPTA) was previously reported to have a profound effect on the induction of lycopene in a wide array of carotenogenic tissues: fruits, roots or the mycelium of certain plants (C. W. Coggins et al., Science 168:1589, 1970). A stimulation of the lycopene pathway was found in Marsh seedless grapefruit, navel orange and Sinton citrangequat, and a concomitant increase in the provitamin A level (γ -carotene) was observed.

Because of the far reaching implications of this work with regard to beneficially controlling the composition of citrus fruits it became important to determine the mechanism of action of CPTA. One question to be answered was whether CPTA acts at the enzyme level or the gene level. For this study the mold *Blakeslea trispora* was chosen because it offered the important advantages of a convenient carotenoid producing plant system which grows rapidly and in large numbers on a small scale. The carotenoid pigments of the mycelia of mated *B. trispora* are essentially hydrocarbon in nature. The mycelia accumulate β -carotene and therefore acquire a yellow-orange color.

When *B. trispora* was treated with CPTA, a response very similar to that observed in citrus fruits was obtained. Lycopene accumulated as the principal pigment with a concomitant increase in γ -carotene formation. Further results indicated that CPTA influences carotenogenesis in multiple ways. The current working hypothesis is that it acts both as an enzyme inhibitor and a gene derepressor. It appears that accumulation of lycopene and γ -carotene is due to inhibition of the cyclase(s) causing a blockage of the cyclization reactions which lead to further metabolism of these carotenoids. It also appears that CPTA derepresses the gene responsible for synthesis of an enzyme or enzymes in the biosynthetic sequence leading to lycopene, thereby stimulating lycopene production. The stimulatory (derepressor) effect of CPTA seems to be more pronounced than its inhibitory effect. These and other results will be considered in relation to the induction of color and provitamin A content of citrus fruits.

SOME WATER MANAGEMENT FACTORS IN A CITRUS PROCESSING PLANT

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Effluents generated by the use of fresh water are exceedingly complex, and those of the food industries are not exceptional from this standpoint. In general, one expects food plant effluents to display substantial oxygen demand and mineralization. Water quality indicators such as oxygen demand (BOD, COD), filterable residue, conductivity, total hardness, etc. are valuable in helping to define the nature of a water quality problem and in helping to judge the effectiveness of control procedures. However, these indicators offer little in terms of pointing toward suitable means for upgrading effluent quality.

A strategy that can be used when confronted with an effluent quality problem follows:

- 1) Develop a detailed understanding of the system that produces the effluent.
- 2) Postulate plausible treatment methods.
- 3) Assign to each candidate method a weight that expresses its suitability.
- 4) Perform appropriate pilot tests.

While the steps enumerated may be arbitrarily separated as they have been here, in practice they will ordinarily interlock in a somewhat complex way. Thus, although the weighing of alternatives might largely consist of data processing, its outcome is likely to depend substantially upon the character of the framework in which the data originate. Furthermore, the nature of the investigational framework will depend not only upon factors of the type mentioned but also the extent to which the problems at hand are correlated with information that exists outside the immediate problem area. With considerations of this sort in mind, work on a citrus plant effluent has recently been initiated at the Pasadena Laboratory.

Several times during the summer of 1971 samples were obtained from the effluent system of a lemon processing plant. The results of this examination tend to substantiate the hope that the knowledge gained will be helpful in upgrading effluent quality.

Experimental work was centered about the technique of bubble flotation. Unhumidified air from the laboratory compressor was introduced into columns of sample specimens through fritted glass discs. Performance was evaluated in terms of either residual solids determined by ashing at 525°C. or chemical

oxygen demand determined by dichromate digestion or both.

Major experimental results follow.

- 1) Effluent displayed significant variation of total mineral content.
- 2) In the experiment devoted to rate evaluation a substantial fraction of the mineral content was rapidly removed.
- 3) Oxygen demand was monitored in the most recent experiment; removal of oxidizable matter exceeded removal of mineral matter.

Certain relationships between work completed and the development of improved water management techniques will be discussed.

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